Appendix 4

Preliminary Cumulative Hazard and Dose-Response Assessment for Organophosphorus Pesticides:

Determination of Relative Potency and Points of Departure for Cholinesterase Inhibition

Appendix 4

The following pages demonstrate an example of the dose-response modeling for two male rat RBC cholinesterase data sets for an artificial organophosphate pesticide, Organophos. The iterative curve fitting procedure was described in detail in the main document and is also given below.

Determination of Potency for a Single Cholinesterase Measurement

Step 1. Each cholinesterase data set was first fit to the exponential function (Equation 1) using generalized least squares regression. Model parameters for *A*, *B,* and *m* were estimated. Confidence limits and standard errors for these estimates were also calculated.

The exponential function used for modeling the effect of the OPs on cholinesterase activity was:

$$y = B + (A - B) \times e^{-m*dose} \qquad \text{Equation 1}$$

where ***y*** is cholinesterase activity,
dose is the dose of OP, in mg/kg/day,
m is the dose scale factor and is the measure of absolute potency,
A is background cholinesterase activity,
and ***B*** is the y-asymptote.

Step 2. If the p-value of the Pearson's Chi-Square statistic was $\geq$ 0.05, model fitting was considered adequate. No doses were dropped.

Step 3. If the p-value of the Pearson's Chi-Square statistic was < 0.05 or the model did not converge, the y-asymptote (i.e., the B-term in Equation 1) was set to 0. The dose-response model was fit again. At this point, if the p-value $\geq$ 0.05, refinements were stopped.

Step 4. If the p-value < 0.05, then high doses were sequentially dropped following by refitting the dose-response function (using B = 0) until 1) the p-value $\geq$ 0.05 or 2) only three dose groups remained (i.e., control and two pesticide treatment groups).

Data set #1 achieved adequate model goodness-of-fit where the A, B, and m (absolute potency) parameters were each estimated using all available data (Step 1 and 2). Data set #2 achieved adequate model goodness-of-fit after B was set to zero (Step 3) and also a single high dose was dropped (Step 4).

DATA SET #1

ORGANOPHOS:360-D:RBC:M:MAIN

Chemical Name: Time of Cholinesterase measurement (days): Sex: Status on study (main study, satellite, replicate, recovery, etc.)
Date and Time of Analysis

MRID identification number;

Mon Jul 16 10:46:21 2001

MRID: MRID1 Guideline: 83-1
Continuous Exponential Model (Decreasing)
Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

Summary of Model Fitting Results

AIC	BIC	logLik
254.4252	258.4081	-123.2126

Coefficients:

	Value	Std.Error	t-value	p-value
A	3757.534534	533.8202254	-0.5622712	5.812703e-01
B	148.968073	16.2512528	-30.3199191	3.059762e-16
m	2.928908	0.2775056	24.8615813	8.322730e-15

STEP 1.
Exponential model parameters with standard error and t-value. *A*, *B,* and *m* estimated .

Correlation:

	A	B	m
A	1.0000000	0.1103358	0.6976685
B	0.1103358	1.0000000	0.3868074
m	0.6976685	0.3868074	1.0000000

Approximate 95% confidence intervals

Coefficients:

	lower	est.	upper
A	2784.389582	3757.534534	5070.793926
B	118.340684	148.968073	187.522043
m	2.398229	2.928908	3.577014

Upper and lower 95% confidence limits on the model parameters

Residual standard error:

lower	est.	upper
848.2141	1130.3679	1694.5843

Degrees of freedom: 20 total; 17 residual

Goodness of Fit

Pearson Chi-Square Statistic: 3.317 with 2 degrees of freedom. P = 0.190

STEP 2.
Model goodness-of-fit. ***p-value $0.05.*** All doses utilized.

	dose	n	chei	Expected	sd	Exp.SD	X2 Resid.
1	0.0	4	4070	3757.5345	720	1130.09647	0.5529890
2	0.6	4	670	771.4542	320	190.17006	-1.0669835
3	1.2	4	290	256.3484	40	65.10368	1.0337859
4	2.4	4	160	152.1634	20	46.24809	0.3388943
5	3.6	4	130	149.0632	40	45.91425	-0.8303808

Doses, sample size, cholinesterase activity (chei), and standard deviations (sd) values extracted from toxicity studies. Expected values, expected standard deviations, and Chi-square residuals from the model fitting.

BMD Computation

BMD = 0.03754: BMDL = 0.03235

Calculation of BMD_{10} and BMDL

Potency Measures

A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity

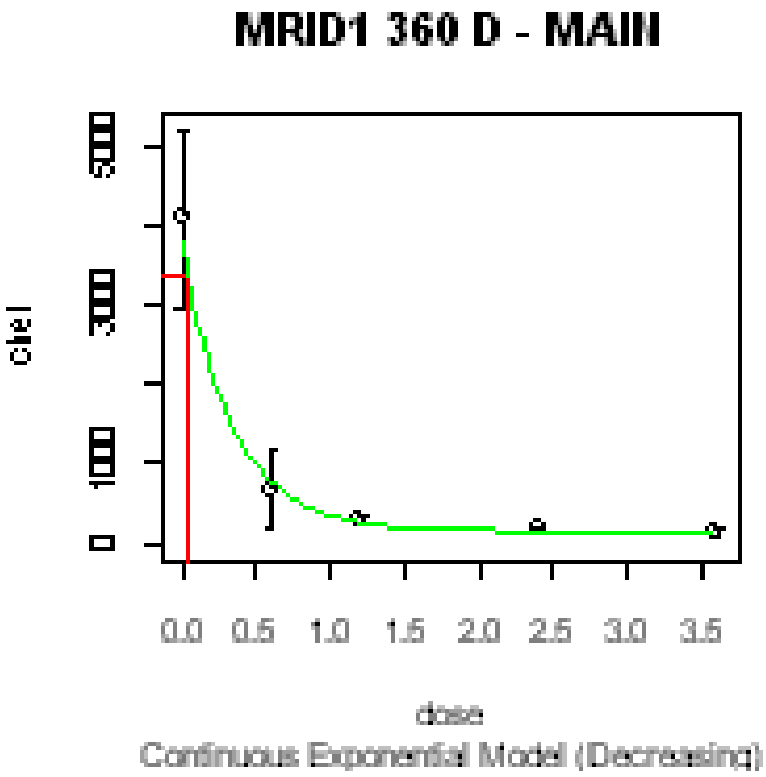
Potency: 2.929
se: 0.2775
var=se^2: 0.07701
Per cent. of background at unit dose: 5.3
Per cent. of background at the highest dose: 0.0026
ED50 (95% CI): 0.2367 (0.1965 , 0.285)

Additional point estimates of potency

ln(Potency) 1.075
se[log(Potency)]: 0.09475
se[log(Potency)]^2: 0.008977

DATA SET #1

Figure 1. Dose-response curve for RBC cholinesterase measured in male rats following exposure to Organophos (MRID #1, 360 day measurement). All available data was used to determine potency.



DATA SET #2

ORGANOPHOS:90-D:RBC:M:MAIN
Mon Jul 16 10:46:24 2001
MRID: MRID2 Guideline: 82-1
Continuous Exponential Model (Decreasing)
Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

Summary of Model Fitting Results

AIC	BIC	logLik
240.9389	244.0293	-116.4695

Coefficients:

	Value	Std.Error	t-value	p-value
A	4315.289352	983.053556	0.01558054	9.878056e-01
B	454.545402	76.191265	-13.40568997	5.475637e-09
m	3.405496	1.252009	6.74063881	1.381962e-05

Correlation:

	A	B	m
A	1.000000000	0.007105104	0.2062495
B	0.007105104	1.000000000	0.5095683
m	0.206249456	0.509568288	1.0000000

STEP 1.
Exponential model parameters with standard error and t-value. *A*, *B,* and *m* estimated.

Approximate 95% confidence intervals

Coefficients:

	lower	est.	upper
A	2637.989462	4315.289352	7059.058596
B	316.453109	454.545402	652.897747
m	1.539018	3.405496	7.535586

Residual standard error:

lower	est.	upper
1425.313	1966.073	3167.428

Degrees of freedom: 16 total; 13 residual

Goodness of Fit

Pearson Chi-Square Statistic: 4.291 with 1 degrees of freedom. P = 0.0383

STEP 2.
Model goodness-of-fit. ***p-value < 0.05.*** All doses utilized.

	dose	n	chei	Expected	sd	Exp.SD	X2 Resid.
1	0.0	4	4300	4315.2894	800	1966.1620	-0.01555248
2	1.0	4	600	582.6849	400	269.2923	0.12859730
3	2.0	4	300	458.7984	140	209.3206	-1.51727461
4	3.5	4	600	454.5711	60	207.1380	1.40417410

BMD Computation

BMD = 0.03481: BMDL = 0.02176

Potency Measures

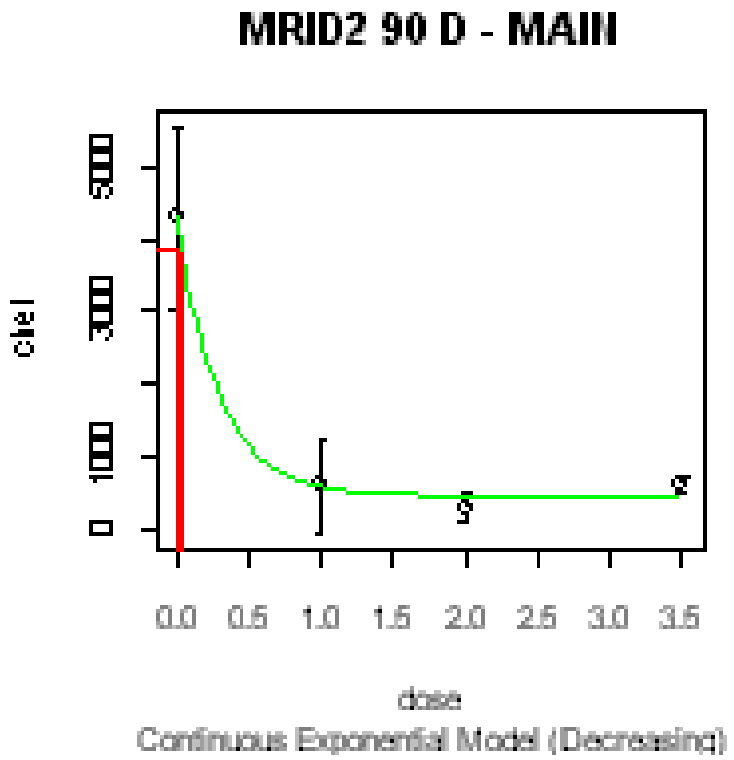
A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity

Potency: 3.405
se: 1.252
var=se^2: 1.568
Per cent. of background at unit dose: 3.3
Per cent. of background at the highest dose: 0.00067
ED50 (95% CI): 0.2035 (0.09901 , 0.4184)

ln(Potency) 1.225
se[log(Potency)]: 0.3676
se[log(Potency)]^2: 0.1352

DATA SET #2

Figure 2. Dose-response curve for RBC cholinesterase measured in male rats following exposure to Organophos (MRID #2, 90 day measurement). All available data was used to determine potency–model goodness-of-fit was *not* adequate.



DATA SET #2

ORGANOPHOS:90-D:RBC:M:MAIN
Mon Jul 16 10:46:26 2001
MRID: MRID2 Guideline: 82-1
Continuous Exponential Model (Decreasing)
Formula: chei = B + (A-B)*exp(-(m*dose)^g)

Variance Function: power

Highest 1 doses dropped from data set.

Alert to reviewer: One dose dropped.

Summary of Model Fitting Results

AIC	BIC	logLik
183.87306	185.32778	-88.93653

Coefficients:

	Value	Std.Error	t-value	p-value
A	3971.101200	617.5707396	-0.5116609	6.199962e-01
m	1.452252	0.1743411	8.8819098	4.659101e-06

STEP 3.
Exponential model parameters with standard error and t-value. *A* and *m* estimated. *B* was set to zero.

Correlation:

	A	m
A	1.0000000	0.6014244
m	0.6014244	1.0000000

Approximate 95% confidence intervals

Coefficients:

	lower	est.	upper
A	2808.166149	3971.101200	5615.638073
m	1.111411	1.452252	1.897620

Residual standard error:

lower	est.	upper
1548.468	2216.158	3889.211

Degrees of freedom: 12 total; 10 residual

Goodness of Fit

Pearson Chi-Square Statistic: 3.727 with 1 degrees of freedom. P = 0.0535

STEP 4.
Model goodness-of-fit. ***p-value $0.05.*** One high dose dropped.

dose	n	chei	Expected	sd	Exp.SD	X2 Resid.
1	0	4	4300	3971.1012	800	1281.3030	0.5133818
2	1	4	600	929.4071	400	438.6051	-1.5020670
3	2	4	300	217.5209	140	150.1397	1.0986974

BMD Computation

BMD = 0.07255: BMDL = 0.06059

Potency Measures

A unit dose (1 mg/kg) would result in 100*exp(-Potency)% of background activity

Potency: 1.452
se: 0.1743
var=se^2: 0.03039
Per cent. of background at unit dose: 23
Per cent. of background at the highest dose: 5.5
ED50 (95% CI): 0.4773 (0.3772 , 0.6039)

ln(Potency) 0.3731
se[log(Potency)]: 0.12
se[log(Potency)]^2: 0.01441

DATA SET #2

Figure 2. Dose-response curve for RBC cholinesterase measured in male rats following exposure to Organophos (MRID #2, 90 day measurement). One high dose dropped–model goodness-of-fit was adequate.